APPENDIX A

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Thomas R. ADAMS et al.	Group Art Unit: 1638
Serial No.: 08/113,561	Examiner: Fox, David T.
Filed: August 25, 1995	Atty. Dkt. No.: DEKM:055US
For: METHOD AND COMPOSITIONS FOR THE PRODUCTION OF STABLY TRANSFORMED, FERTILE MONOCOT PLANTS AND CELLS THEREOF	

DECLARATION OF VIRGINIA URSIN UNDER 37 C.F.R. § 1.132

Assistant Commissioner for Patents
Washington, D.C. 20231

I, VIRGINIA URSIN HEREBY DECLARE AS FOLLOWS:

1. I have been employed by Calgene Inc. and Monsanto Company since 1989, currently with the position of Project Lead Lipid Technologies. Monsanto Company is the parent company of wholly owned subsidiaries Calgene Inc. and Monsanto Company.

2. I hold a Ph.D. in Genetics from University of California. I have been conducting research in the area of agricultural biotechnology since 1987.

3. I understand that the Patent and Trademark Office Examiner in charge of assessing the patentability of the referenced patent application has rejected the claims as not being supported by adequate information in the specification to show that introduction of a heterologous fatty acid desaturase in maize would result in an altered grain composition trait that would render such a maize plant identifiable over the corresponding untransformed maize plants which do not comprise the heterologous gene.

4. Therefore, I am providing the present Declaration to submit further data that demonstrates that heterologous expression of fatty acid desaturase genes in maize alters the fatty acid profile of transgenic plants in a predictable and consistent manner that renders them identifiable over corresponding non-transgenic plants.

5. *Vector Construction and Transformation of Maize*

A binary vector was constructed to express a Δ15-desaturase and a Δ6-desaturase in maize embryo and aleurone tissue. This construct was prepared with the globulin promoter (see, e.g., Table 3, Regulatory Sequence 123 of Pat. Appl. Serial no. 08/113,561) driving expression of a mutagenized *Neurospora crassa* Δ15 desaturase and a *Mortierella alpina* Δ6 desaturase (SEQ ID NO:21, bp 71-1444) (U.S. Pat. No. 6,075,183). The *M. alpina* Δ6 desaturase was cloned into a globulin expression cassette shuttle vector, pMON67624, resulting in pMON82809. The mutagenized *N. crassa* Δ15 desaturase was cloned into a globulin expression cassette vector, pMON67624, resulting in pMON82810.

The two globulin desaturase expression cassettes were then cloned into the pMON30167 1T maize binary vector containing the CP4 marker gene for glyphosate resistance. The first expression cassette containing the *M. alpina* Δ6 desaturase was cloned into pMON30167, resulting in pMON82811. The second expression cassette containing the mutagenized *N. crassa* Δ15 desaturase was then cloned into pMON82811, resulting in a maize transformation construct designated pMON82812. The resulting vector was introduced into maize via *Agrobacterium tumefaciens*-mediated transformation as known to one of skill in the art, *e.g.*, U.S. Patent Nos. 5,591,616 and 6,603,061.

6. *Fatty Acid Analysis*

The fatty acid composition of single immature kernels of plants transformed with vector pMON82812 was determined by lyophilizing maize kernels and extracting the kernels with toluene and 5.0 % (wt/vol) sulfuric acid in methanol, followed by heat treatment. Following the heat treatment, the reaction mixture was extracted with heptane followed by aqueous sodium chloride (10% wt/vol). After partitioning at room temperature, the organic phase was analyzed by GLC (Hewlett Packard model 6890 (120volt) equipped with a split/splitless capillary inlet (250°C) and a flame ionization detector (270°C). The column was a Supelco 24077 (0.25 mm

od. x 15 m length) with a 0.25 µm bonded polyethylene glycol stationary phase. The fatty acid methyl esters are identified by retention time comparison to commercial standards. Qualitative weight percent compositions are calculated as area percents of identified peaks.

The data in Table 1 below demonstrate fatty acid profiles for kernels of transgenic maize expressing the mutagenized *Neurospora crassa* Δ15 desaturase and *Mortierella alpina* Δ6 desaturase of pMON82812 and accumulating SDA (18:4) and GLA (18:3), which are not seen in untransformed lines. These events also demonstrate increased accumulation of ALA (18:3) and decreased accumulation of LA (18:2). Of 180 seeds tested, 67 contained SDA and GLA, 25 contained GLA but not SDA, and 88 were wild type with respect to GLA and SDA.

TABLE 1: Fatty Acid Analysis of Single Immature Maize Kernels Expressing SDA and/or GLA

Pedigree	Event	Gen	Oleic (18:1)	LA (18:2)	GLA (18:3)	ALA (18:3)	SDA (18:4)
ZM_S103121:@.	ZM_S103121	R1	21.5	25.54	1.22	28.45	2.08
ZM_S103121:@.	ZM_S103121	R1	21.21	29.49	1.32	24.92	1.83
ZM_S103121:@.	ZM_S103121	R1	18.93	32.23	1.85	24.58	1.62
ZM_S103121:@.	ZM_S103121	R1	19.99	29.81	1.46	26.36	1.36
ZM_S103435/LH244	ZM_S103435	F1	19.56	34.49	0.7	23.78	0.88
ZM_S103121:@.	ZM_S103121	R1	17.24	35.26	1.3	23.99	0.8
ZM_S103435/LH244	ZM_S103435	F1	19.78	36.27	0.61	22.2	0.6
ZM_S103435/LH244	ZM_S103435	F1	19.61	34.73	0.56	23.59	0.58
ZM_S103432:@.	ZM_S103432	R1	19.44	33.05	0.83	25.89	0.57
ZM_S103121:@.	ZM_S103121	R1	18.99	33.41	0.64	25.39	0.55
ZM_S103432:@.	ZM_S103432	R1	19.72	33.14	0.88	24.82	0.52
ZM_S103121:@.	ZM_S103121	R1	18.08	35.7	0.97	22.92	0.49
ZM_S103435/LH244	ZM_S103435	F1	19.34	35.22	0.54	23.56	0.47
ZM_S103435/LH244	ZM_S103435	F1	17.95	36.96	0.54	22.88	0.43
ZM_S103433/LH244	ZM_S103433	F1	19.18	43.84	0.22	14.98	0.36
ZM_S103437/LH244	ZM_S103437	F1	20.26	42.75	0.69	14.73	0.36
ZM_S103435/LH244	ZM_S103435	F1	19.75	41.59	0.58	17.1	0.35
ZM_S103437/LH244	ZM_S103437	F1	21.04	42.89	0.67	14.03	0.35
ZM_S103110:@.	ZM_S103110	R1	18.62	37.51	0.57	22.51	0.34
ZM_S103432:@.	ZM_S103432	R1	19.5	38.38	0.77	20.26	0.34

ZM_S103437/LH244	ZM_S103437	F1	19.18	44.68	0.64	14.1	0.32
ZM_S103435/LH244	ZM_S103435	F1	19.11	38.92	0.38	20.6	0.3
ZM_S103432:@.	ZM_S103432	R1	17.99	40.62	0.74	19.34	0.3
ZM_S103432:@.	ZM_S103432	R1	17.93	40.5	0.78	19.26	0.3
ZM_S103432:@.	ZM_S103432	R1	19.55	38.86	0.75	19.66	0.29
ZM_S103110:@.	ZM_S103110	R1	19.84	48.95	0.52	9.9	0.28
ZM_S103110:@.	ZM_S103110	R1	19.21	37.14	0.41	22.57	0.28
ZM_S103432:@.	ZM_S103432	R1	19.33	38.28	0.72	20.5	0.28
ZM_S103435/LH244	ZM_S103435	F1	19.68	42.85	0.59	15.62	0.28
LH244/ZM_S103123	ZM_S103123	F1	18.01	40.3	0.36	20.22	0.27
ZM_S103110:@.	ZM_S103110	R1	19.51	42.17	0.54	17.37	0.27
ZM_S103437/LH244	ZM_S103437	F1	20.08	45.06	0.58	12.99	0.26
ZM_S103435/LH244	ZM_S103435	F1	19.5	42.99	0.45	15.9	0.25
LH244/ZM_S103123	ZM_S103123	F1	19.41	43.27	0.41	15.9	0.25
ZM_S103168/LH244	ZM_S103168	F1	18.78	44.78	1.32	14.48	0.24
ZM_S103110:@.	ZM_S103110	R1	17.95	41.13	0.58	19.46	0.23
ZM_S103432:@.	ZM_S103432	R1	17.81	40.06	0.67	20.1	0.22
ZM_S103435/LH244	ZM_S103435	F1	19.26	44.01	0.51	14.7	0.22
ZM_S103436/LH244	ZM_S103436	F1	19.88	45.18	0.49	13.4	0.22
ZM_S103168/LH244	ZM_S103168	F1	20.19	43.05	1.06	15.11	0.21
ZM_S103110:@.	ZM_S103110	R1	19	46.61	0.46	13.13	0.21
LH244/ZM_S103431	ZM_S103431	F1	18.73	37.53	0.27	22.93	0.2
ZM_S103099/LH244	ZM_S103099	F1	19.32	38.76	0.54	20.49	0.2
LH244/ZM_S103123	ZM_S103123	F1	20.26	36.51	0.28	22.25	0.19
ZM_S103433/LH244	ZM_S103433	F1	19.4	43.5	0.21	15.38	0.19
ZM_S103168/LH244	ZM_S103168	F1	19.44	44.9	0.99	13.94	0.19
ZM_S103168/LH244	ZM_S103168	F1	20.59	44.07	0.79	13.9	0.19
ZM_S103110:@.	ZM_S103110	R1	19	46.29	0.51	13.61	0.19
ZM_S103437/LH244	ZM_S103437	F1	19.9	45.07	0.51	13.25	0.19
ZM_S103436/LH244	ZM_S103436	F1	19.97	45.59	0.41	13.06	0.19
ZM_S103168/LH244	ZM_S103168	F1	20.92	49.2	0.58	9.12	0.18
LH244/ZM_S103431	ZM_S103431	F1	18.27	37.66	0.32	22.96	0.18
ZM_S103103/LH244	ZM_S103103	F1	19.19	46.83	0.7	12.21	0.18
ZM_S103436/LH244	ZM_S103436	F1	18.34	48.08	0.37	11.98	0.18
ZM_S103433/LH244	ZM_S103433	F1	19.71	43.32	0.22	15.12	0.17
ZM_S103436/LH244	ZM_S103436	F1	19.16	47.28	0.37	11.95	0.17
LH244/ZM_S103431	ZM_S103431	F1	18.78	37.05	0.27	23.34	0.16

ZM_S103099/LH244	ZM_S103099	F1	18.66	40.7	0.45	19.85	0.16
ZM_S103433/LH244	ZM_S103433	F1	19.78	43.09	0.22	15.39	0.16
ZM_S103430/LH244	ZM_S103430	F1	19.92	42.81	0.69	16.07	0.15
ZM_S103437/LH244	ZM_S103437	F1	20.17	46.64	0.48	10.88	0.15
LH244/ZM_S103123	ZM_S103123	F1	19.87	40.51	0.23	18.3	0.14
ZM_S103430/LH244	ZM_S103430	F1	19.25	43.68	0.52	15.4	0.13
ZM_S103436/LH244	ZM_S103436	F1	19.49	47.11	0.31	11.99	0.12
ZM_S103103/LH244	ZM_S103103	F1	19.77	47.5	0.37	11.33	0.12
ZM_S103432:@.	ZM_S103432	R1	18.68	42.32	0.42	17.36	0.11
ZM_S103103/LH244	ZM_S103103	F1	19.85	47.51	0.35	11.33	0.1
ZM_S103168/LH244	ZM_S103168	F1	17.33	51.87	1.13	7.55	0
ZM_S103099/LH244	ZM_S103099	F1	18.41	40.56	0.67	19.94	0
ZM_S103433/LH244	ZM_S103433	F1	17.88	52.22	0.62	7.58	0
ZM_S103097/LH244	ZM_S103097	F1	18.77	47.41	0.61	12.28	0
ZM_S103430/LH244	ZM_S103430	F1	18.35	46.05	0.57	13.91	0
ZM_S103110:@.	ZM_S103110	R1	18.48	45.05	0.48	15.43	0
ZM_S103436/LH244	ZM_S103436	F1	19.8	46.28	0.42	13.34	0
ZM_S103103/LH244	ZM_S103103	F1	20.11	47.5	0.42	11	0
ZM_S103099/LH244	ZM_S103099	F1	18.38	41.21	0.42	19.45	0
ZM_S103099/LH244	ZM_S103099	F1	18.62	41.08	0.4	19.14	0
ZM_S103103/LH244	ZM_S103103	F1	19.9	48.09	0.38	10.89	0
LH244/ZM_S103123	ZM_S103123	F1	18.47	45.81	0.37	14.52	0
ZM_S103103/LH244	ZM_S103103	F1	19.8	48.52	0.32	10.36	0
LH244/ZM_S103123	ZM_S103123	F1	18.45	46.48	0.32	13.79	0
LH244/ZM_S103123	ZM_S103123	F1	19.27	41.97	0.24	17.97	0
ZM_S103433/LH244	ZM_S103433	F1	19.84	43.54	0.23	14.61	0
ZM_S103433/LH244	ZM_S103433	F1	19.68	43.73	0.22	15.15	0
ZM_S103433/LH244	ZM_S103433	F1	18.85	43.9	0.22	15.03	0
ZM_S103433/LH244	ZM_S103433	F1	19.72	44.98	0.19	13.42	0
ZM_S103434/LH244	ZM_S103434	F1	20.56	43.62	0.14	15.03	0
ZM_S103434/LH244	ZM_S103434	F1	19.8	44.48	0.14	14.84	0
ZM_S103434/LH244	ZM_S103434	F1	19.85	44.6	0.13	14.68	0
ZM_S103434/LH244	ZM_S103434	F1	18.53	45.5	0.12	14.86	0
ZM_S103434/LH244	ZM_S103434	F1	20.17	44.8	0.12	14.43	0
ZM_S103434/LH244	ZM_S103434	F1	19.38	45.86	0.12	13.73	0

7. The results of the above studies demonstrated that expression of a fatty acid desaturase gene in maize alters the fatty acid profile in a manner that renders the transgenic plants identifiable over corresponding non-transgenic plants. The results further confirm that the alteration of fatty acid profiles in maize occurs in a predictable manner that is consistent with the enzymatic activity of the fatty acid desaturase that is introduced into a given maize plant.

8. I hereby declare that all statements made herein of my knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

13 October, 2004
Date

Virginia Ursin